

What Is Claimed Is:

1. An oligonucleotide useful as a primer for inducing mutagenesis in a complementarity determining region (CDR) of an immunoglobulin light chain gene, said oligonucleotide having 3' and 5' termini and comprising:

a) a nucleotide sequence at said 3' terminus capable of hybridizing to a first framework region of an immunoglobulin gene;

b) a nucleotide sequence at said 5' terminus capable of hybridizing to a second framework region of an immunoglobulin gene; and

c) a nucleotide sequence between said 3' and 5' termini according to the formula:

[NNK]_n,

wherein N is independently any nucleotide, K is G or T, n is 3 to about 24, said 3' and 5' terminal nucleotide sequences having a length of about 6 to 50 nucleotides, or an oligonucleotide having a sequence complementary thereto.

2. The oligonucleotide of claim 1 wherein said 5' terminus has the nucleotide sequence 5'-TATACTGTCAGCAGTAT-3' (SEQ ID NO 26) or 5'-GATTTTGCAGTGTATTACTGTCAGCAGTAT-3' (SEQ ID NO 27), or an oligonucleotide having a sequence complementary thereto.

3. The oligonucleotide of claim 1 wherein said 3' terminus has the nucleotide sequence 5'-ACTTTCGGCGGAGGGACCAAGGTGGAG-3' (SEQ ID NO 28) or 5'-ACTTTCGGCGGAGGGACC-3' (SEQ ID NO 29), or an oligonucleotide having a sequence complementary thereto.

4. The oligonucleotide of claim 1 wherein n is 4, 5, 6, 10 or 16.

5. The oligonucleotide of claim 1 wherein said immunoglobulin is human.

6. The oligonucleotide of claim 1 wherein said CDR is CDR3.

5 7. The oligonucleotide of claim 1 according to the formula: 5'-
GATTTTGCAGTGTATTACTGT [NNK]_n TTCGCGGAGGGACCAAGGTGGAG-
3' (SEQ ID NO 12), or an oligonucleotide having a sequence complementary thereto.

10 8. An oligonucleotide useful as a primer for inducing mutagenesis in a complementarity determining region (CDR) of an immunoglobulin light chain gene, said oligonucleotide having 3' and 5' termini and comprising:

15 a) a nucleotide sequence at said 3' terminus capable of hybridizing to a first framework region of an immunoglobulin gene;

b) a nucleotide sequence at said 5' terminus capable of hybridizing to a second framework region of an immunoglobulin gene; and

20 c) a nucleotide sequence between said 3' and 5' termini according to the formula:

[MNN]_n,

25 wherein N is independently any nucleotide, M is A or C, n is 3 to about 24, said 3' and 5' terminal nucleotide sequences having a length of about 6 to 50 nucleotides, or an oligonucleotide having a sequence complementary thereto.

30 9. The oligonucleotide of claim 8 wherein said 5' terminus has the nucleotide sequence 5'-
GTTCCACCTTGGTCCCTTGGCCGAA-3' (SEQ ID NO 30), or an oligonucleotide having a sequence complementary thereto.

35 10. The oligonucleotide of claim 8 wherein said 3' terminus has the nucleotide sequence 5'-

ACAGTAGTACACTGCAAAATC-3' (SEQ ID NO 31), or an oligonucleotide having a sequence complementary thereto.

11. The oligonucleotide of claim 8 wherein n is 8, 10 or 16.

12. The oligonucleotide of claim 8 wherein said immunoglobulin is human.

13. The oligonucleotide of claim 8 wherein said CDR is CDR3.

14. A method for producing an antibody combining site in a polypeptide comprising inducing mutagenesis in a complementarity determining region (CDR) of an immunoglobulin light chain gene which comprises amplifying a CDR portion of the immunoglobulin gene by polymerase chain reaction (PCR) using a PCR primer oligonucleotide, said oligonucleotide having 3' and 5' termini and comprising:

a) a nucleotide sequence at said 3' terminus capable of hybridizing to a first framework region of an immunoglobulin gene;

b) a nucleotide sequence at said 5' terminus capable of hybridizing to a second framework region of an immunoglobulin gene; and

c) a nucleotide sequence between said 3' and 5' termini according to the formula:

$[NNK]_n$,

wherein N is independently any nucleotide, K is G or T, and n is 3 to about 24, said 3' and 5' terminal nucleotide sequences having a length of about 6 to 50 nucleotides, or an oligonucleotide having a sequence complementary thereto.

15. The method of claim 14 wherein said 5' terminus has the nucleotide sequence 5'-

TATACTGTCAGCAGTAT-3' (SEQ ID NO 26) or 5'-

GATTTTGCAGTGTATTACTGTCAGCAGTAT-3' (SEQ ID NO 27), or

an oligonucleotide having a sequence complementary thereto.

16. The method of claim 14 wherein said 3' terminus has the nucleotide sequence 5'-

5 ACTTTCGGCGGAGGGACCAAGGTGGAG-3' (SEQ ID NO 28) or 5'-
ACTTTCGGCGGAGGGACC-3' (SEQ ID NO 29), or an
oligonucleotide having a sequence complementary
thereto.

10 17. The method of claim 14 wherein n is 4, 5, 6,
10 or 16.

18. The method of claim 14 wherein said immunoglobulin is human.

19. The method of claim 14 wherein said CDR is CDR3.

15 20. The method of claim 14 according to the
formula: 5'-
GATTTTGCAGTGTATTACTGT[NNK]₁₀TTCGGCGGAGGGACCAAGGTGGAG-
3' (SEQ ID NO 12), or an oligonucleotide having a
sequence complementary thereto.

20 21. The method of claim 14 wherein said
immunoglobulin light chain gene includes a sequence
having the sequence characteristics of the light chain
shown in SEQ ID NO 2 or in SEQ ID NO 62.

25 22. The method of claim 14 wherein said
immunoglobulin light chain gene has the sequence
characteristics of the light chain gene in ATCC
Accession No. 75408.

23. The method of claim 14 that further comprises the steps of:

30 a) isolating the amplified CDR to form a
library of mutagenized immunoglobulin light chain
genes;

35 b) expressing the isolated library of
mutagenized light chain genes in combination with one
or more heavy chain genes to form a combinatorial

antibody library of expressed heavy and light chain genes; and

c) selecting species of said combinatorial library for the ability to bind a presel

of claim 23 wherein said c

chain genes is a lib

antibody

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antibody library of expressed heavy and light chain genes; and

c) selecting species of said combinatorial antibody library for the ability to bind a preselected antigen.

24. The method of claim 23 wherein said one of said immunoglobulin heavy chain genes is a library of genes for producing an antibody comprising a variable region inducing mutagenesis in a complementary region (CDR) of a heavy chain comprising

24. The method of claim 23 wherein the antibody library for the antibody selection is a library of more immunoglobulin heavy chain genes is a library of more immunoglobulin heavy chain genes.

antigen. The method more immunoglobulin heavy chain genes.

24. A method for producing a site in a polypeptide comprising inducing a complementarity determining region (CDR) in an immunoglobulin light chain gene which comprises amplifying a CDR portion of the immunoglobulin gene by polymerase chain reaction (PCR) using a PCR primer oligonucleotide, said oligonucleotide having 3' and 5' termini and comprising:

a) a nucleotide sequence at said 3' capable of hybridizing to a first framework immunoglobulin gene;

b) a nucleotide sequence at said 5' capable of hybridizing to a second framework immunoglobulin gene; and

c) a nucleotide sequence between said

complement
oglobulin light
ifying a CDR portion of
ymerase chain reaction (PCR) to
igonucleotide, said oligonucleotide
termini and comprising:
a) a nucleotide sequence at said 3'
terminus capable of hybridizing to a first framework
region of an immunoglobulin gene;
b) a nucleotide sequence at said 5'
terminus capable of hybridizing to a second framework
region of an immunoglobulin gene; and
c) a nucleotide sequence between said
regions of the formula:

b) a nucleotide sequence capable of hybridizing to a region of an immunoglobulin gene; and

c) a nucleotide sequence between said terminus capable of hybridizing to a region of an immunoglobulin gene;

d) a nucleotide sequence according to the formula:

[MNN]_n

wherein M is independently any nucleotide, N is A or G, n is about 24, said 3' and 5' terminal nucleotides having a length of about 6 nucleotides each, and said nucleotide sequence having a self-complementary sequence.

b) a nucleotide sequence capable of hybridizing with an immunoglobulin gene, and 5' termini according to the formula:

[MNN]_n

wherein N is independently any nucleotide, M is A or C, n is 3 to about 24, said 3' and 5' terminal nucleotides, or an oligonucleotide having a sequence complementary thereto.

2. A method of claim 25 wherein said 5' nucleotide sequence 5' - 3' (SEQ ID NO 30), or a complementary

30 wherein N is 1 to about 10, C, n is 3 to about 10, and the nucleotide sequences have nucleotides, or an oligonucleotide complementary thereto.

26. The method of claim 25 wherein the terminus has the nucleotide sequence 5'-GTTCCACCTTGGTCCCTTGGCCGAA-3' (SEQ ID NO 30), or an oligonucleotide having a sequence complementary thereto.

27. The method of claim 25 wherein said 3' terminus has the nucleotide sequence 5'-ACAGTAGTACACTGCAAAATC-3' (SEQ ID NO 31), or an oligonucleotide having a sequence complementary thereto.

28. The method of claim 25 wherein n is 8, 10 or 16.

29. The method of claim 25 wherein said immunoglobulin is human.

30. The method of claim 25 wherein said CDR is CDR3.

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31. The method of claim 25 wherein said immunoglobulin light chain gene includes a sequence having the sequence characteristics of the light chain shown in SEQ ID NO 2 or in SEQ ID NO 62.

32. The method of claim 25 wherein said immunoglobulin light chain gene has the sequence characteristics of the light chain gene in ATCC Accession No. 75408.

33. The method of claim 25 that further comprises the steps of:

a) isolating the amplified CDR to form a library of mutagenized immunoglobulin light chain genes;

b) expressing the isolated library of mutagenized light chain genes in combination with one or more heavy chain genes to form a combinatorial antibody library of expressed heavy and light chain genes; and

c) selecting species of said combinatorial antibody library for the ability to bind a preselected antigen.

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34. The method of claim 33 wherein said one or more immunoglobulin heavy chain genes is a library of heavy chain genes.